

A novel and sensitive method for recognition and indirect determination of Al^{III} in biological fluid based on the quenching of resonance Rayleigh scattering intensities of “Al^{III}-EV-DNA” complexing system

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Abstract

A novel method for recognition and indirect determination of Al^{III} by using biological molecules has been established based on the quenching of RRS intensity. In the weak acidic medium, the reaction of ethyl violet (EV) and DNA would result in great enhancement of RRS intensity. However, the presence of Al^{III} would lead to the decrease of the RRS intensity owing to the competition coordination of Al with DNA. The decreased intensity of RRS is directly proportional to the concentration of Al^{III} in the range of $(0.1\text{--}2.5) \times 10^{-6}$ and $(0.30\text{--}4.5) \times 10^{-5}$ M, respectively. The method has high sensitivity and its detection limit (3σ) is 3.6×10^{-8} M. The characteristics of RRS spectra of the system, the optimum conditions of the reaction, and the reaction mechanism have been investigated. The method can recognize Al^{III} selectively owing to its strong binding to the phosphate backbone of DNA, and has been applied to the determination of Al^{III} concentration in synthetic biological samples with satisfactory results. Therefore, the proposed method is promising as an effective means for selective recognition and sensitive determination *in situ* of Al^{III}. Furthermore, this study would contribute to further understanding of the biological significance of Al neurotoxicity.

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1. Introduction

Resonance Rayleigh scattering (RRS) is a special elastic scattering produced when the wavelength of Rayleigh scattering (RS) is located at or close to its molecular absorption band. In this case, the frequency of the electromagnetic wave absorbed by the electron is equal to its scattering frequency. Owing to the intensive absorption of light energy of the electron, re-scattering takes place. Therefore, the scattering intensity is enhanced several orders of magnitude compared with single RS and no longer obeys the Rayleigh law of $I \propto \lambda^{-4}$ [1,2]. RRS also shows the characteristics of the scattering spectrum as well as that of the electronic absorption spectrum. Compared to that of a single RS technique, it not only has high sensitivity and better selectivity, but also can provide plenty of new

information concerning molecular structure, size, form, charge, distribution, state of combination, and so on. In recent years, the technique is mainly applied to the study and determination of some biological macromolecules, which is based on the fact that the aggregation of chromophores (e.g. porphyrin, acridine orange, methyl violet, ethyl violet, etc.) on biological macromolecule can give rise to strong RRS [3–11]. Liu et al. found that the form of ion-association can also lead to a great enhancement of RRS intensity. Based on the characteristic, a series of metal ions have been selectively determined by using RRS technique [12–17]. Compared with other analytical methods, RRS method possesses distinct advantages of high speed, convenience and sensitivity, and can be accomplished with a common fluorescence spectrometer by using inexpensive and safe reagents. So the RRS technique is anticipated to become a new and highly sensitive technique for determination of biological macromolecules and inorganic ions.

Aluminum (Al) is the most abundant metal in the earth's crust. The environmental and biological effects of Al have been

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increasingly studied in recent years [18,19]. Most of the studies have reported that elevated concentrations of Al in natural waters and soils due to the increased acidic precipitation are toxic to fish, marine bacteria and plants. Certain human illnesses may be related to Al [20,21]. For these reasons, an increasing attention has been paid to the study and determination of Al. The most commonly employed analytical techniques for Al determination are graphite furnace atomic absorption spectrometry and inductively coupled plasma emission spectrometry, but both of these techniques are relatively expensive [22]. Spectrophotometric method and fluorimetric method are often used for determination of Al. The former method, based on the color reaction between Al and some organic reagents, is precise and reliable, but it often suffers from the disadvantage of low sensitivity or procedural complications [23,24]. The fluorometric method is based on the quench or enhancement of the fluorescence of various compounds after they interact with nucleic acids. Although the fluorimetric method has high sensitivity, it is only limited to the fluorescent active substance, and some of them suffer from the serious interference of co-existing ions or long reaction time (60 min) [25–27]. Therefore, it is important to develop a simple, rapid and sensitive method to determine Al.

In this paper, based on the strong binding of Al^{III} to the phosphate backbone of DNA, we have developed a new, selective method for indirect determination of trace amounts of Al^{III} based on the quenching of the RRS intensity by using biological molecules. In the experiment we discovered that in weak acidic medium, the reaction of ethyl violet (EV) and DNA would result in great enhancement of RRS intensity and its maximum scattering peak is located at 498 nm. However, the presence of Al^{III} will lead to the decrease of the RRS intensity owing to the competition coordination of Al with DNA. In a certain range, the decreased intensity of RRS is directly proportional to the concentration of Al^{III} . The method has high sensitivity and its detection limit (3σ) can be down to 3.6×10^{-8} M. The characteristics of RRS spectra of the system, the optimum conditions of the reaction and the influencing factors have been investigated. The method with better selectivity is successfully applied to the determination of Al^{III} concentration in synthetic samples. The study of the effect of other metal ions on RRS intensity of the EV-DNA system shows further that the method can selectively recognize Al. Therefore, DNA can be used as a sensitive probe to detect Al^{III} by using an EV indicator. What is more, this study not only provides a new field for the determination of metal ions with biological macromolecules by using RRS technique, but also contributes to further understanding of the biological significance of aluminum neurotoxicity.

2. Experimental

2.1. Reagents and apparatus

A Shimadzu RF-850 spectrofluorometer (Kyoto, Japan) was used for measuring the RRS intensity at given wavelength using a 1-cm path length with the slit (EX/EM) of 10.0 nm/10.0 nm.

The stock solution of calf thymus DNA (ctDNA) was prepared by dissolving commercially purchased ctDNA (Hua Mei Institute of Biochemistry, China) in water, which was kept at 4°C with gentle shaking occasionally. The concentration of DNA was determined according to the absorbance at 260 nm after establishing that the absorbance ratio A_{260}/A_{280} was in the range 1.80–1.90. The stock solution of the DNA was kept in a refrigerator at 4°C and its working concentration was 6.0×10^{-5} M. The working solution of ethyl violet (EV) was prepared by dissolving the crystallized product of EV (Shanghai Chemical Reagent Company, China) in water. Its concentration was 2.0×10^{-4} M.

The stock solution of Al (0.02 M) was prepared by dissolving super-purity Al powder (Shanghai Chemical Reagent Company, 99.99%) in 1:10 HCl solution. It was diluted to 1.0×10^{-4} M when constructing the working curve. 0.05 M Tris-HCl buffer solution was used to control the pH value of the interacting system. All reagents were of analytical reagent grade and were used without further purification. Doubly distilled water was used throughout.

2.2. Procedure

EV solution (0.5 ml) was added into a 10-ml calibrated flask firstly, and then 1.0 ml buffer solution, 1.0 ml ctDNA solution, and certain amount of Al^{III} standard solution were added accordingly. This solution was diluted to 10 ml with doubly distilled water and mixed thoroughly under room temperature. Twenty minutes later, the RRS spectra was recorded with synchronous scanning at $\lambda_{\text{em}} = \lambda_{\text{ex}}$ and the RRS intensity was measured, I_{RRS} for the reaction product and I_0 for the reagent blank at the maximum scattered wavelength, $\Delta I = |I_{\text{RRS}} - I_0|$.

3. Results and discussion

3.1. Resonance Rayleigh scattering spectrum

Fig. 1 shows the RRS spectra of the EV-ctDNA- Al^{III} system from 250 to 800 nm. It can be seen that both RRS intensities of ctDNA and EV are very weak. In addition, the experimental results by adsorption spectrum also indicate that the interactions of Al^{III} with EV as well as with DNA are weak (figures are not shown), although Al^{III} can bind strongly with the phosphate groups on the DNA backbone [28]. However, the formation of EV-ctDNA complex will lead to great enhancement of RRS intensity and its maximum scattering peak is located at 498 nm, which is similar to the phenomenon reported in the literature [10,11]. In the presence of Al^{III} , the scattering intensity of the maximum scattering peak decreases owing to the competition coordination of Al^{III} with DNA. Therefore, it can be concluded that the reaction of EV and DNA is the only reason leading to the enhancement of RRS intensity, and the competition coordination of Al^{III} with DNA leads to the decrease of RRS intensity. The absorption spectrum of EV-ctDNA system is similar to the phenomenon reported in the literature [11], from which we can conclude that the electrostatic force is primarily responsible for the binding of the cationic

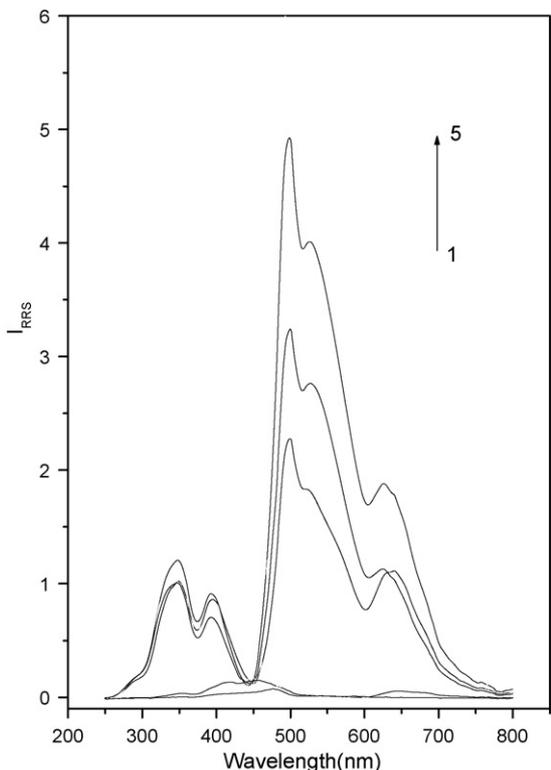


Fig. 1. Resonance Rayleigh scattering spectra of EV-ctDNA- Al^{III} system. (1) ctDNA (6.0×10^{-6} M; pH 5.6); (2) EV (1.0×10^{-5} M); (3–5) EV-ctDNA- Al^{III} system (EV, 1.0×10^{-5} M; ctDNA, 6.0×10^{-6} M; pH 5.6). Concentration of Al^{III} : (3) 2.0×10^{-5} M; (4) 5.0×10^{-6} M; (5) 0 M.

dye to negatively charged phosphate group on nucleic acid [10,28,29].

3.2. Optimization of experimental conditions

The variation of pH value greatly influences the RRS intensities of the system. It affects the charge distribution of EV molecule, the behavior of DNA and the binding ability of the system (see Fig. 2A). It can be seen in Fig. 3A that when the pH value is 5.5, ΔI reaches the maximum value. When the pH is lower or higher than this value, the intensity of ΔI will decrease. Therefore, pH 5.5 is selected as optimum acidity for determination of nucleic acids.

The effect of ctDNA concentration on the RRS intensity also has been investigated (see Fig. 2B). The result shows that the decrease of RRS intensity is most obvious when adding 1.0 ml 6.0×10^{-5} M ctDNA to the solution. Under the con-

ditions, the optimal dye concentration is 1.0×10^{-5} M (see Fig. 2C).

The stability of the system was tested and the result is shown in Fig. 2D.

The effect of ionic strength on the RRS intensity is investigated with various concentrations of NaCl. Ionic strength has an obvious effect on the RRS intensity of ctDNA-EV system (see Fig. 2E). Since in high ionic strength the electrostatic force between Na^{I} and the negative charged groups on nucleic acids is strengthened, it is unfavorable for the combination of EV with negative charged phosphate group on nucleic acids, the reaction is blocked.

3.3. Calibration curve, linear range and detection limit

Under the optimum conditions, the ΔI values of the complexes are measured at their maximum scatter wavelength, and the calibration graphs of ΔI against concentration of Al^{III} are constructed (see Fig. 3). The correlation parameters are presented in Table 1. It is found from the figure that, when the mole ratio of Al^{III} to DNA is near to 2, there is an obvious inflection point, which may be that a kind of cooperative binding process occurs [30,31]. The mechanism for this complexing effect is unknown right now and deserves further studying in the future. ΔI is directly proportional to the concentration of Al^{III} in the range of $(0.1\text{--}2.5) \times 10^{-6}$ and $(0.30\text{--}4.5) \times 10^{-5}$ M, respectively. The linear regression equations are $\Delta I_{\text{RRS}} = 0.362 + 4.18 \times 10^6 c$ ($R = 0.9957$) and $\Delta I_{\text{RRS}} = -0.929 + 3.62 \times 10^5 c$ ($R = 0.9975$), respectively. The former is much more sensitive than the latter. The relative standard deviation for 5.0×10^{-6} M Al is 4.5% ($n = 5$). The method has high sensitivity, and the detection limit of the system calculated as three times the standard deviation for the blank buffer solution, is 3.6×10^{-8} M.

3.4. Selectivity of the method and the determination of aluminum in synthetic samples

The selectivity of the method was inspected by determining of 5.0×10^{-6} M Al^{III} in the presence of a series of foreign substances. The results show that 100-fold EDTA, and F^- , 50-fold DL-glutamic acid, L-leucine, L-aspartic acid, L-tyrosine, PO_4^{3-} and Mg^{II} , 20-fold Zn^{II} , Ni^{II} , Co^{II} , 10-fold Fe^{III} , and Cr^{III} , 5-fold Hg^{II} and Cd^{II} have no interference. Large amounts of Na^{I} , K^{I} , Cl^- , NO_3^- , and SO_4^{2-} do not interfere. The proposed method is applied to the determination of trace alu-

Table 1
The results for the determination of Al in synthetic samples

Sample	Added value ($\times 10^{-6}$ M)	Main additives ^a	Found value ($\times 10^{-6}$ M)	Recovery ($n = 5, \%$)
No.1	5.0	$\text{Hg}^{\text{II}} + \text{Cu}^{\text{II}} + \text{Cr}^{\text{III}} + \text{Ca}^{\text{II}} + \text{Zn}^{\text{II}} + \text{Cd}^{\text{II}} + \text{Na}^{\text{I}} + \text{L-leucine} + \text{D-fructose}$	5.2	104
No. 2	5.0	$\text{Fe}^{\text{III}} + \text{Hg}^{\text{II}} + \text{Cu}^{\text{II}} + \text{Cr}^{\text{III}} + \text{Ni}^{\text{II}} + \text{Mg}^{\text{II}} + \text{Cd}^{\text{II}} + \text{Na}^{\text{I}} + \text{K}^{\text{I}} + \text{EDTA} + \text{lactic acid}$	4.8	96.0
No. 3	5.0	$\text{Fe}^{\text{III}} + \text{Zn}^{\text{II}} + \text{Ca}^{\text{II}} + \text{Cr}^{\text{III}} + \text{Mg}^{\text{II}} + \text{Na}^{\text{I}} + \text{K}^{\text{I}} + \text{PO}_4^{3-} + \text{F}^-$	5.3	106

^a (in 10^{-7} M): Na^{I} , K^{I} : 50; Ca^{II} , Ni^{II} , Cu^{II} , Hg^{II} , PO_4^{3-} : 6.0; Mg^{II} , Ba^{II} : 5.0; Cr^{III} , Zn^{II} : 2.0; Fe^{III} : 0.56; Pb^{II} : 0.2; L-leucine, lactic acid: 10; D-fructose, EDTA, F^- : 20.

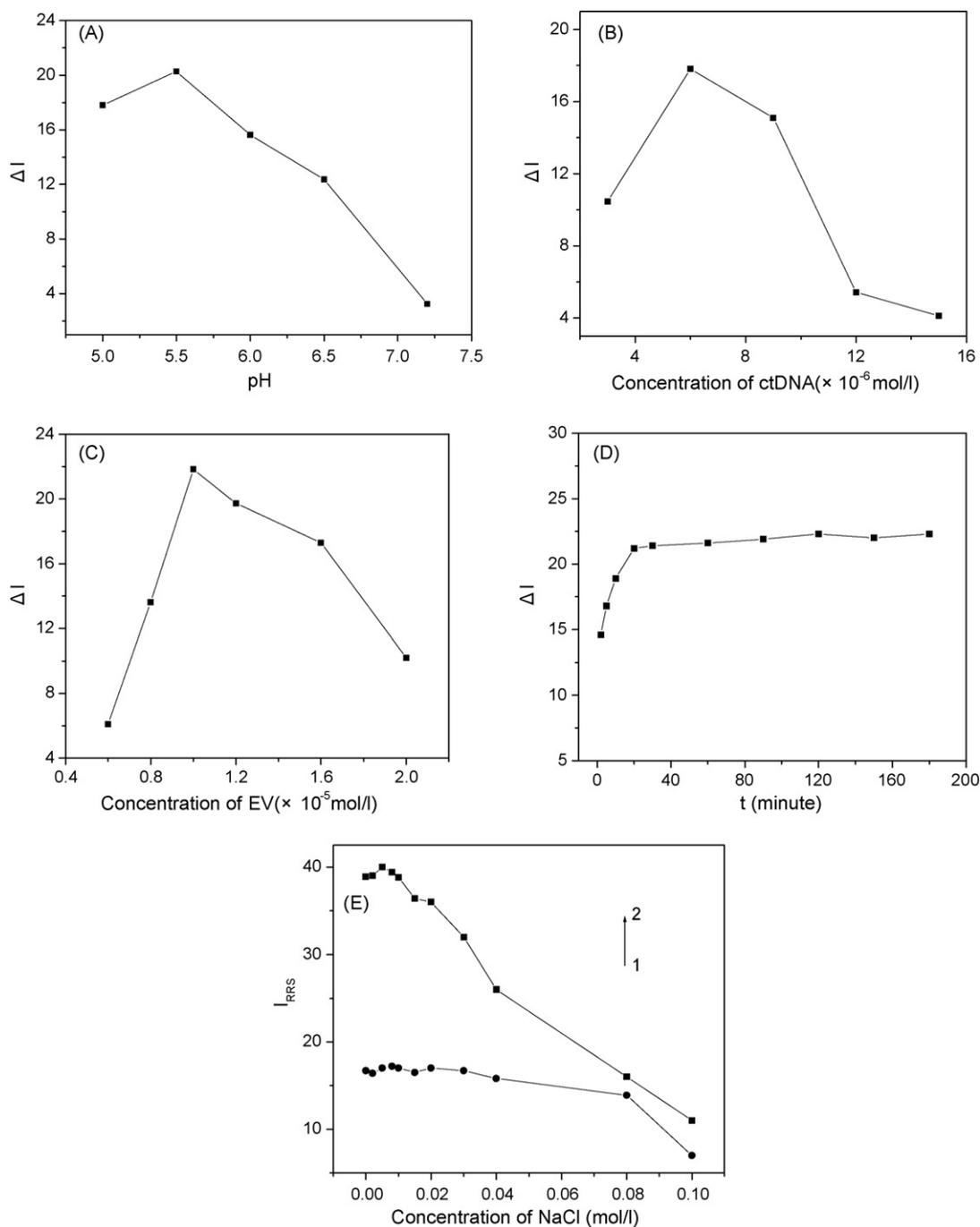


Fig. 2. (A) Effect of pH on the intensity of RRS. (B) Effect of DNA concentration on the intensity of RRS. (C) Effect of dye concentration on the intensity of RRS. (D) Stability of the system. (E) Effect of ionic strength on the intensity of RRS. (1) EV-ctDNA- Al^{III} system; (2) EV-ctDNA system. Concentrations: EV, 1.0×10^{-5} M; ctDNA, 6.0×10^{-6} M; Al^{III} , 5.0×10^{-6} M; pH 5.6.

minimum in three synthetic samples with satisfactory results (see Table 1).

3.5. The specific recognition of Al^{III} by DNA

We have investigated the reactions between DNA and other metal ions using EV as an indicator, and plot the calibration graphs of ΔI against concentration of metal ions (see Table 2). It shows that the addition of metal ion will lead to the decrease of RRS intensity, but the decreased extents are different for dif-

ferent metal ions, which reflect the different binding ability of DNA with different metal ions. The results show that, among them, the effect of Al^{III} is strongest. The effects of Na^I , K^I and Mg^{II} on EV-ctDNA system are small (ΔI values hardly change). It can be interpreted that the binding abilities of these metal ions with DNA are weak. However, it is interesting that, although some heavy metal ions (such as Hg^{II} and Cd^{II}) and Cu^{II} have strong binding abilities with DNA, they also slightly affect the RRS intensity of EV-ctDNA system. The reason may be that the binding sites for the metal ions on DNA are mainly the bases,

Table 2
Effect of other metal ions on the EV-ctDNA system

Concentration ($\times 10^{-4}$ M)	0	0.02	0.05	0.08	0.1	0.2	0.3	0.5	0.8	1.0	2.0	2.5	3.0
ΔI (Na ^I)	0	1.2	1.2	1.1	1.4	1.5	1.4	1.8	1.6	2.1	2.4	1.8	1.3
ΔI (K ^I)	0	0.8	1.3	1.2	1.5	1.4	1.7	2.4	1.8	2.3	1.8	2.6	3.0
ΔI (Mg ^{II})	0	1.1	2.3	1.8	2.1	3.2	2.6	3.1	3.6	4.2	4.8	5.4	7.6
ΔI (Cu ^{II})	0	1.2	2.0	2.3	2.6	3.1	2.8	3.4	4.6	5.8	6.4	7.8	9.6
ΔI (Hg ^{II})	0	1.1	0.8	0.9	1.2	1.4	0.9	1.3	1.6	1.4	1.5	1.6	1.8
ΔI (Cd ^{II})	0	0.8	0.9	1.3	1.2	1.5	1.7	1.4	1.8	1.6	1.4	1.2	1.5

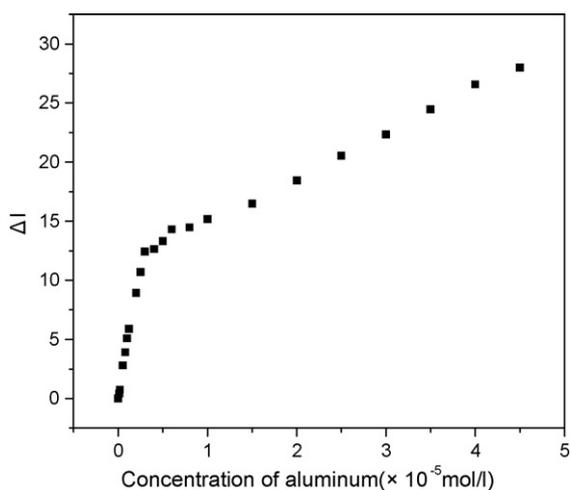


Fig. 3. Quenching of RRS intensity of EV-ctDNA system with the addition of Al^{III}. Concentrations: EV, 1.0×10^{-5} M; ctDNA, 6.0×10^{-6} M; pH 5.6.

which differ from that of EV [32,33]. This phenomenon not only explains the reason that most of metal ions do not interfere with the determination of Al^{III} in the proposed method, but also indicate DNA can recognize Al^{III} selectively by using EV as an indicator.

4. Conclusion

In recent years, there has been a growing interest in the development of some optical biosensors for recognition and selective determination of metal ions. They often focus on the use of conformational change of proteins, peptides and DNA structure, to detect metal ions in biological sample as alternative way [34–36]. When an indicator is attached to the biological macromolecules, this conformational change will affect its optical and electrochemical signal, such as fluorescence, etc.

In this paper, based on the quenching of RRS signal, we developed a new, selective method for indirect determination of trace amounts of Al^{III} by biological macromolecules. In weak acidic medium, the interaction of Al^{III} with nucleic acid has been investigated using EV as an indicator by RRS method and absorption spectroscopy. According to the experimental results and the previous literature [10,28,29], it can be concluded that Al^{III} has great affinity for phosphate ligating sites, and the electrostatic forces are primarily responsible for the binding of EV to negatively charged phosphate groups on nucleic acid, in which the binding ability of Al^{III} with the phosphate group on the DNA backbones is stronger than EV. Therefore, the competition reac-

tion between EV and Al for the DNA is the main reason for the decrease of RRS intensity. In a certain range, the decrease of scattering signal is directly proportional to the concentration of Al^{III}. Based on this characteristic, a novel method for the determination of trace amount of Al^{III} by using EV as an indicator has been established. The proposed method is simple and sensitive (D.L. = 5.0×10^{-8} M). What's more, owing to the reaction group of nucleic acid with Al differs from many metal ions, it can selectively recognize Al^{III} from them. Therefore, the method is promising to be an effect means for selective recognition and sensitive determination of Al^{III} in the real sample. Furthermore, the study will also be helpful for further understanding of the detailed mechanism of Al neurotoxicity and the biochemical process of Al^{III} *in vivo* on a molecular level [37].

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References

- [1] S.P. Liu, H.Q. Luo, N.B. Li, Z.F. Liu, M. Li, W.X. Zheng, *Anal. Chem.* 73 (2001) 3907.
- [2] S.G. Stanton, R. Pecora, B.S. Hudson, *J. Chem. Phys.* 75 (1981) 5615.
- [3] R.F. Pasternack, P.J. Collings, *Science* 269 (1995) 935.
- [4] R.F. Pasternack, C. Bustamante, P.J. Collings, A. Giannetto, E. Gibb, *J. Am. Chem. Soc.* 115 (1993) 5393.
- [5] C.Z. Huang, K.A. Li, S.Y. Tong, *Anal. Chem.* 69 (1997) 514.
- [6] C.Z. Huang, Y. Liu, Y.H. Wang, H.P. Guo, *Anal. Biochem.* 321 (2003) 236.
- [7] X.F. Long, S.P. Bi, X.C. Tao, Y.Z. Wang, H. Zhao, *Spectrochim. Acta Part A* 60 (2004) 455.
- [8] X.F. Long, S.P. Liu, L. Kong, Z.F. Liu, S.P. Bi, *Talanta* 63 (2004) 279.
- [9] R.T. Liu, J.H. Yang, C.X. Sun, L. Li, X. Wu, Z.M. Li, C.S. Qi, *Chem. Phys. Lett.* 376 (2003) 108.
- [10] Y. Liu, C.Q. Ma, K.A. Li, F.C. Fu, S.Y. Tong, *Anal. Biochem.* 268 (1999) 187.
- [11] T.J. Li, H.X. Shen, *Chem. J. Chin. Univ.* 19 (1998) 1570.
- [12] S.P. Liu, Z.F. Liu, M. Li, N.B. Li, H.Q. Luo, *Fresenius J. Anal. Chem.* 368 (2000) 848.
- [13] S.P. Liu, Z.F. Liu, H.Q. Luo, *Anal. Chim. Acta* 407 (2000) 255.
- [14] S.P. Liu, Q. Liu, Z.F. Liu, C.Z. Huang, *Anal. Chim. Acta* 379 (1999) 53.
- [15] X.F. Long, S.P. Bi, H.Y. Ni, X.C. Tao, N. Gan, *Anal. Chim. Acta* 501 (2004) 89.
- [16] Q.E. Cao, Y.K. Zhao, X.J. Yao, Z.D. Hu, Q.H. Xu, *Spectrochim. Acta Part A* 56 (2000) 1319.
- [17] S.P. Liu, Z.F. Liu, C.Z. Huang, *Anal. Sci.* 14 (1998) 799.
- [18] N. Clarke, L.G. Danielsson, A. Sparen, *Pure Appl. Chem.* 68 (1996) 1597.

- [19] K. Pyrzynska, S. Gucer, E. Bulska, *Water Res.* 34 (2000) 359.
- [20] J. Liu, X.L. Wang, G. Chen, N. Gan, S.P. Bi, *Analyst* 126 (2001) 1404.
- [21] D.R.C. Mclachlan, C. Bergeron, J.E. Smith, D. Boomer, S.L. Rifat, *Neurology* 46 (1996) 401.
- [22] M. Du, C.W. Huie, *Anal. Chim. Acta* 443 (2001) 269.
- [23] R.L. Benson, P.J. Worsfold, F.W. Sweeting, *Anal. Chim. Acta* 238 (1990) 177.
- [24] O. Royset, *Anal. Chem.* 59 (1987) 899.
- [25] H.B. Li, F. Chen, *Anal. Bioanal. Chem.* 368 (2000) 501.
- [26] C. Brach-Papa, B. Coulomb, J.L. Boudenne, V. Cerda, F. Theraulaz, *Anal. Chim. Acta* 457 (2002) 311.
- [27] J.M. Escriche, F.H. Hernandez, *Analyst* 110 (1985) 287.
- [28] R.Y. Zhang, Y. Liu, D.W. Pang, R.X. Cai, Y.P. Qi, *Anal. Sci.* 18 (2002) 761.
- [29] B.H. Yun, S.H. Jeon, T.S. Cho, S.Y. Yi, U. Sehlstedt, S.K. Kim, *Biophys. Chem.* 70 (1998) 1.
- [30] C.Z. Huang, Y.F. Li, S.Y. Deng, S.Y. Liu, *Bull. Chem. Soc. Jpn.* 72 (1999) 1501.
- [31] Y.F. Li, C.Z. Huang, M. Li, *Anal. Chim. Acta* 452 (2002) 285.
- [32] Z. Hossain, F. Huq, J. Inorg. Biochem. 90 (2002) 85.
- [33] V.A. Sorokin, I.V. Sysa, V.A. Valeev, G.O. Gladchenko, M.V. Degtyar, Y.P. Blagoi, *J. Mol. Struct.* 408 (1997) 233.
- [34] K. Flora, J.D. Brennan, *Anal. Chem.* 70 (1998) 4505.
- [35] T. Nguyen, Z. Rosenzweig, *Anal. Bioanal. Chem.* 374 (2002) 69.
- [36] V.A. Romoser, P.M. Hinkle, A. Persechini, *J. Biol. Chem.* 272 (1997) 13270.
- [37] C. Exley, *Aluminum and Alzheimer's Disease—the Science that Describes the Link*, Elsevier, London, 2001.